PATENT COOPERATION TREATY

147

REC'D 05 AUG 2005

PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

6/10

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2005/000763	24.03.2005	26.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
QIAGEN AS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Reuter, U

Telephone No. +31 70 340-1036

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/000763

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/000763

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22
	No:	Claims	23-25
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-25
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V.

1 Reference is made to the following documents:

D1: WO 2004/001015 A (PEL-FREEZ CLINICAL SYSTEMS, LLC; WANG, LU; XIANGJUN, LIU) 31 December 2003 (2003-12-31)

D2: WO 01/90419 A (VARIAGENICS, INC; STANTON, VINCENT, P., JR) 29 November 2001 (2001-11-29)

D3: ALDERBORN A ET AL: "Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 10, no. 8, August 2000 (2000-08), pages 1249-1258, XP002218192 ISSN: 1088-9051

2 **NOVELTY** (Art. 33(2) PCT)

2.1 D1 discloses a kit (p. 33, par. 2) that is suitable for determining one or more nucleic acid sequences that comprises one or more sequencing primers complementary to a region of common sequence, implicitly the enzymes that are necessary to perform a pyrosequencing reaction (example 3, p. 18) and fluorescently labelled nucleotides (p. 29, second paragraph). D1 thus discloses all the technical features of claims 23-25 in combination.

2.2 D2 discloses a kit that is suitable for determining one or more nucleic acid sequences that comprises one or more sequencing primers complementary to a region of common sequence and one or more labelled nucleotides (p. 65). The label can be fluorescent (p. 63). D2 thus discloses all the technical features of claims 23-24 in combination.

2.3 D3 discloses one or more sequencing primers complementary to a region of common sequence (p. 1251), one or more fluorescently labelled nucleotides (p. 1256, right col.) and the enzymes that are necessary to perform a pyrosequencing reaction (p. 1250 left col.). D3 thus discloses all the technical features of claims 23-25 in combination.

2.4 In the light of D1, D2 and D3 claims 23-25 are not novel in the sense of Art. 33(2)

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3 **INVENTIVE STEP** (Art. 33(3) PCT)

3.1 Regarding the subject matter of claim 1 D2 is regarded as closest prior art: D2 discloses a method for detecting haplotypes. In the method of D2 one allele is isolated and the enriched nucleic acid is sequenced (p. 13 last par.- p. 14). To isolate the allele the method of D2 comprises the steps of contacting a preparation with an oligonucleotide primer complementary to at least a portion of the first region of common sequence, under conditions to hybridise the primer thereto; contacting the preparation with a labelled nucleotide, biotinylated dNTP, (p. 65) that is complementary to a template nucleotide in the first region of dissimilar sequence in the target nucleic acid under conditions to incorporate said labelled nucleotide into the primer hybridised to the target nucleotide. The incorporated labelled nucleotide is used to separate the target allele from the non-target allele (p. 65-p. 66 l. 2). The target allele is subjected to a sequencing reaction (p. 51).

3.2 The difference of D2 to the subject matter of claim 1 is that in the method of claim 1 the allele specific labelled primer is used in a subsequent sequencing reaction in order to determine the sequence of at least a portion of the labelled or non-labelled sequencing products. This allows an easier detection of haplotypes.

3.3 Confronted with the problem of having to provide an easier method to detect haplotypes neither D2 nor any other document of the cited prior art teaches the person skilled in the art to modify the method of D2 in order to achieve the method of claim 1.

3.4 However the subject matter of claim 1 cannot be regarded as involving an inventive step because the claim extends to methods that do not solve the technical problem and are therefore not inventive:

3.4.1 If the sequencing reaction of step (b) is performed with the non-labelled primer non-labelled sequencing products will be generated that are derived from the target and the non-target sequence since the primer binds to and can be extended along the

target sequence as well as the non-target sequence. If subsequently in step (d) the sequence of the non-first labelled sequencing products is determined it represents a mixture of target and non-target sequences.

3.4.2 If in the method fluorescent labels are used and the sequencing is performed with pyrosequencing (see description and claim 22) the person skilled in the art is confronted with the problem that in the sequencing method, which is performed without the use of a label and in which the release of pyrophosphate is detected during the synthesis of the sequencing product (D3 p. 1250 left col.), he has to find a way to detect the fluorescent label attached to the primer as well. Additionally he has to find a way to differenciate between the pyrophosphate that is released during the sequencing reaction of the target sequence and the pyrophosphate that is released during the sequencing reaction of the non-target sequence that takes place with the unlabelled primer.

3.4.3 If the first label is biotin and the biotin is bound to a solid phase (claim 4), given the fact that the biotin is bound to the nucleotide that forms the 3' end of the primer, said primer cannot be extended in a sequencing reaction using a polymerase.

3.5 Consequently independent claim 1 does not fulfil the requirements of inventive step of Art. 33(3) PCT.

3.6 The same reasoning applies mutatis mutandis for the independent claims 18 and 22 (see 3.4.2) as well as for the dependent claims 2-17 and 19-21.

3.7 In the light of the reasoning given above claims 1-25 do not fulfil the requirements of inventive step of Art. 33(3) PCT.